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Pure Red Cell Aplasia With Thymoma: Evidence of T-Cell Clonal Disorder

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Pure red cell aplasia (PRCA) sometimes accompanies thymoma. Herein, we report a PRCA patient with thymoma with a clonal disorder of T cells. A 55-year-old man presented with anemia and anterior mediastinum tumor. The laboratory study revealed hemoglobin 8.2 g/dl; leukocytes 15.8 × 10^9/L with 76.5% neutrophils, 20.0% lymphocytes, and reticulocytes 0.0%. Bone marrow aspirate smears and biopsy sections revealed normal myeloid and megakaryocyte differentiation and contained no erythroid precursors. We made the diagnosis of PRCA. The size of the lymphocytes was small without any granules in the cytoplasm. The surface marker of peripheral blood mononuclear cells demonstrated increased $CD2^+$, $CD3^+$, $CD4^-$, and $CD8^+$ populations. The mediastinal tumor was resected and a thymoma diagnosed. A monoclonal rearrangement of T-cell receptor (TCR)- β-chain gene was found using Southern blot analysis of the mononuclear cells in both peripheral blood and thymoma. Treatment with prednisolone, thymectomy, and cyclophosphamide exerted no beneficial effect. After initiation of the Cyclosporin A therapy, the patient developed reticulocytosis. This PRCA case seems to present a neoplastic proliferation of $CD8^+$ T cells in peripheral blood and thymus with a monoclonal rearrangement of the TCR− β-chain gene. Am. J. Hematol. 54:324–328, 1997.

Key words: pure red cell aplasia; thymoma; T-cell receptor-β; Cyclosporin A

INTRODUCTION

Pure red cell aplasia (PRCA) is characterized by anemia, reticulocytopenia, and severe erythroid hypoplasia of bone marrow. Acquired secondary PRCA has been described in conjunction with numerous conditions such as thymoma, hematological malignancies, nonhematological solid tumor, infections, drug and exposure to chemicals, hemolytic anemias, collagen disease, pregnancy, severe renal failure and severe nutritional deficiencies [1]. Chronic parvovirus B-19 infection in the immunodeficiency state is most common cause of PRCA [2,3]. Thymoma is accompanied by parathymic syndromes in more than 40% of the patients [4], the most common association being with myasthenia gravis, PRCA, and hypogammaglobulinemia [5]. However, PRCA with thymoma has not been reported to be a common clonal disorder. We suggest that some patient with PRCA with thymoma is a clonal disorder of T cells. We report such a case herein.

CASE REPORT

In March 1994, a 55-year-old man, was admitted to our hospital because of developed dyspnea on exertion and fever. The chest roentgenogram revealed an anterior mediastinal mass and an infiltrated shadow in the left lower lung field. Pneumonia was diagnosed and antibiotics (Cefotiam 4 g/day and Tobramicin 180 mg/day) were given. After 2 weeks of antibiotics therapy, the pneumonia was cured. He presented an interior mediastinal mass, but without lymphoadenopathy, hepatosplenomegaly or skin lesions. Computed tomography (CT) revealed an infiltrating tumor mass located in the anterior mediastinum. The laboratory study disclosed RBC 2.62 × 10^{12}/L; hemoglobin 8.2 g/dl; hematocrit

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TABLE I. Surface Marker Analysis of Mononuclear Cells

	Peripheral blood	Thymoma	Peripheral blood (remission with CyA)
CD1	0 (%)	42.2(%)	0.5(%)
CD2	93.6	95.6	97.0
CD3	87.9	62.4	91.9
CD4	20.4	50.3	24.0
CD5	83.0	93.5	73.9
CD7	82.2	84.8	87.4
CD8	67.9	80.6	64.9
CD10	0.8	11.0	0.9
CD11b	19.9	6.1	12.2
CD13	4.0	1.5	1.1
CD14	1.4	2.0	1.1
CD16	6.9	1.0	3.2
CD19	0	1.2	0.1
CD20	0.1	1.6	0.6
CD25	0.9	2.7	1.0
CD33	1.0	2.5	1.0
CD56	9.2	2.5	5.3
CD57	15.1	4.9	9.2
HLA-DR	9.2	8.1	13.5

25.6%; leukocytes 15.8 × 10^9/L with 76.5% neutrophils, 20.0% lymphocytes, 3.5% monocytes; platelets 32.3 × 10^{10}/L; and reticulocytes 0.0%. Bone marrow aspirate smears and biopsy sections revealed normal myeloid and megakaryocyte differentiation and contained no erythroid precursors. Chromosomal analysis showed no abnormalities. We made the diagnosis of PRCA. The size of the lymphocytes was small without any granules in the cytoplasm. Surface markers of peripheral blood mononuclear cells demonstrated increased in the $CD2^+$, $CD3^+$, $CD4^-$, and $CD8^+$ populations (Table I). Laboratory findings revealed hypogammaglobulinemia (0.26 g/dl). The patient's serum did not show circulating autoantibodies, antibodies to human T-cell leukemia virus-1 (ATL-1) or human immunodeficiency virus (HIV). He then administered prednisolone (1 mg/kg/day). After 1 month, when no therapeutic effect had been obtained, the prednisolone was discontinued. In May 1994, the mediastinal tumor was resected and a thymoma diagnosed. Histological examination of the thymoma revealed encapsulated multinodular neoplasms composed of spindle-shaped epithelial cells (Fig. 1). The surface markers of thymoma mononuclear cells demonstrated an increase in the $CD2^+$,

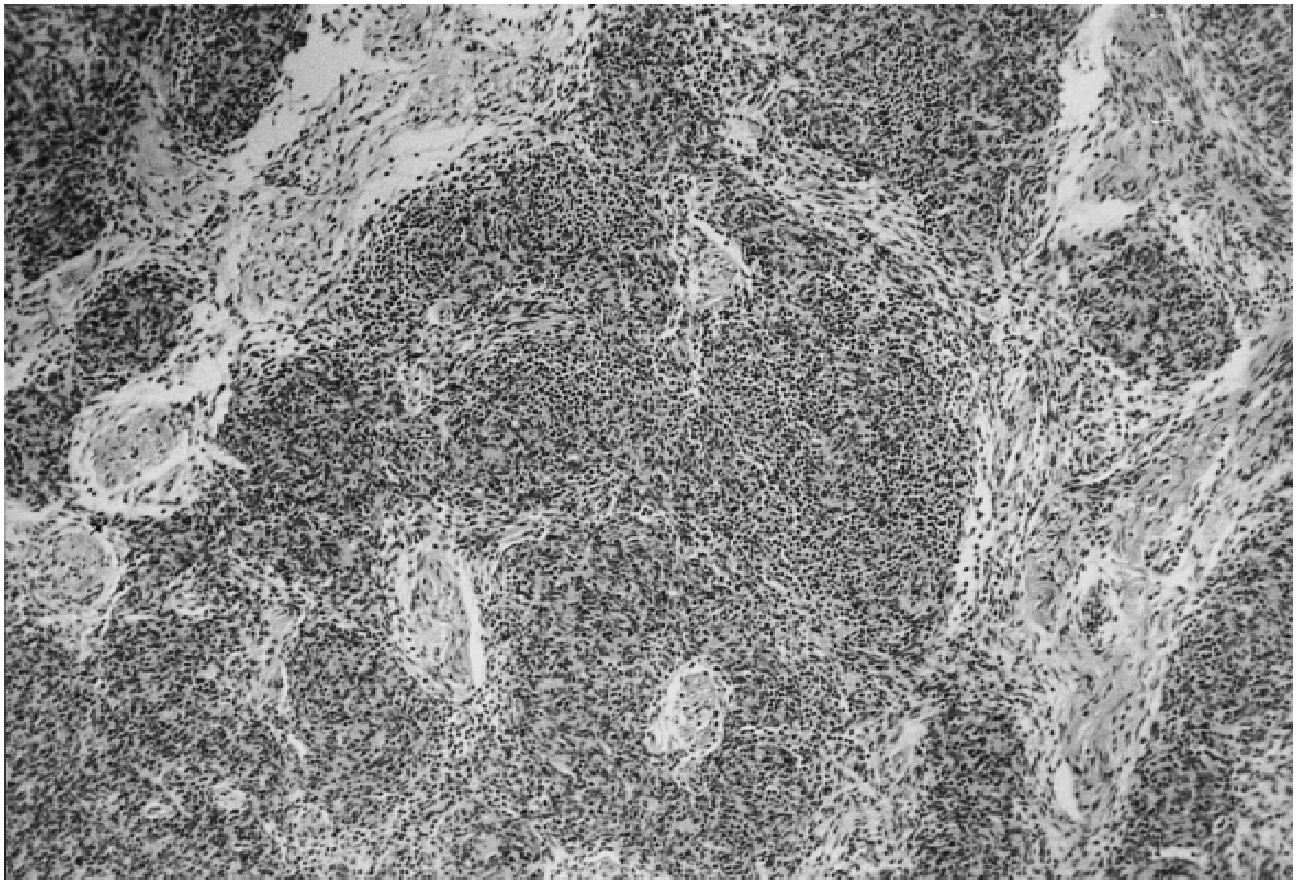

Fig. 1. Thymoma showing abundant small lymphocytes mixed with larger, rounded epithelial cells. (Hematoxylin and eosin, ×100.)

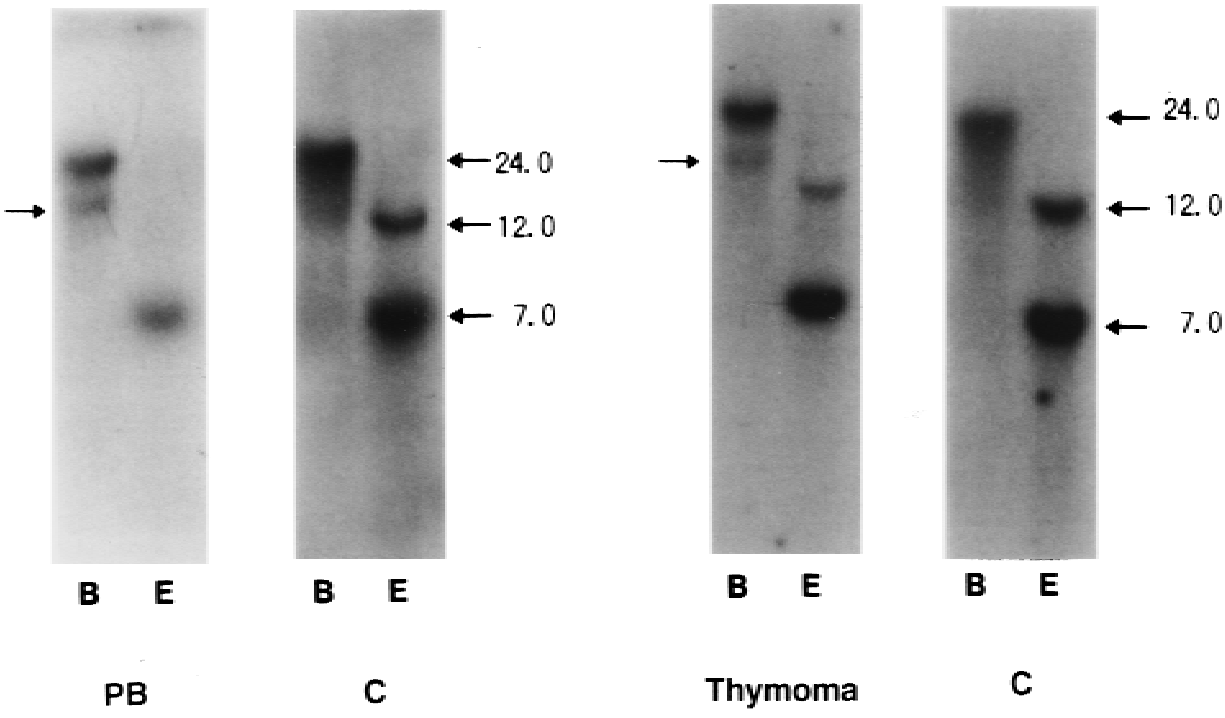

Fig. 2. Gene analysis. DNA samples (5 μg) were digested with *Bam*HI and *Eco*RV. Southern blot analysis of DNA with TCR C β_1 probe was performed. Lines on the right side indicate the germline position. 24.0 kb is the germline position for *Bam*HI; 12.0 kb and 7.0 kb are the germline positions for *Eco*RV. The position of rearranged fragment is marked by an arrow, the control (C) human placental DNA exhibits germline configuration. B, *Bam*HI; E, *Eco*RV; PB, peripheral blood; C, control DNA.

$CD3^+$, $CD4^+$, and $CD8^+$ population (Table I). A monoclonal rearrangement of T-cell receptor (TCR)-β chain gene was found using Southern blot analysis [6] of the mononuclear cells in both peripheral blood and thymoma (Fig. 2). After thymectomy, the $CD2^+$, $CD3^+$, $CD4^-$, $CD8^+$ lymphocytosis and monoclonal rearrangement of the TCR-β chain gene in peripheral blood were unchanged. Since the treatment with prednisolone and the thymectomy exerted no beneficial effect, the patient was then treated with cyclophosphamide (100 mg/day) for 7 months. This resulted in repeated infection without erythropoietic recovery. He needed three packs of RBC transfusions per month. The cyclophosphamide was stopped and therapy with Cyclosporin A (CyA) at 6 mg/kg/day was begun in April 1995. Two weeks after initiation of CyA therapy, the patient developed reticulocytosis (Fig. 3). Seven months after initiation of CyA, the dose of CyA was decreased by 1 mg/kg/month. At 1 mg/kg/day of CyA, reticulocytopenia recurrenced. The patient needed blood transfusion once again. Three weeks after the dose of CyA was increased back to 6 mg/kg/day, the patient again developed reticulocytosis again. During the remission state of PRCA with CyA, the numbers of white blood cells (WBC) and $CD2^+$, $CD3^+$, $CD4^-$, $CD8^+$ lymphocytes were unchanged (Table I).

In this PRCA case, neoplastic proliferation of $CD8^+$ T cells had occurred in peripheral blood and thymus with a monoclonal rearrangement of the TCR-β-chain gene. All treatment was approved by the Investigational Review Board of Tokyo Women's Medical College, and written consent was always obtained. Bone marrow aspirates, peripheral blood, and thymus tissue were obtained after informed consent according to the guidelines of the Investigational Review Board of Tokyo Women's Medical College.

DISCUSSION

In PRCA cases associated with hematological malignancy, the PRCA has been reported to be mediated by B [7] or T cells [8,9]. We report a PRCA patient with thymoma whose case suggest that the thymoma caused by a clonal disorder of T cells. There are several reports that thymoma is associated with peripheral T-cell lymphocytosis. Griffin et al. [10] reported a case of thymoma with T-cell lymphocytosis; the mononuclear cells from peripheral blood, thymoma, and pleural fluid formed rosettes with sheep erythrocytes, and were concluded to be T cells. Jager et al. [11] reported a patient with thymoma and hypogammaglobulinemia. Peripheral lymphocytes showed the surface phenotype of $CD3^+$ 8^+. After thymectomy, peripheral $CD3^+$ 8^+ lymphocytosis was un-

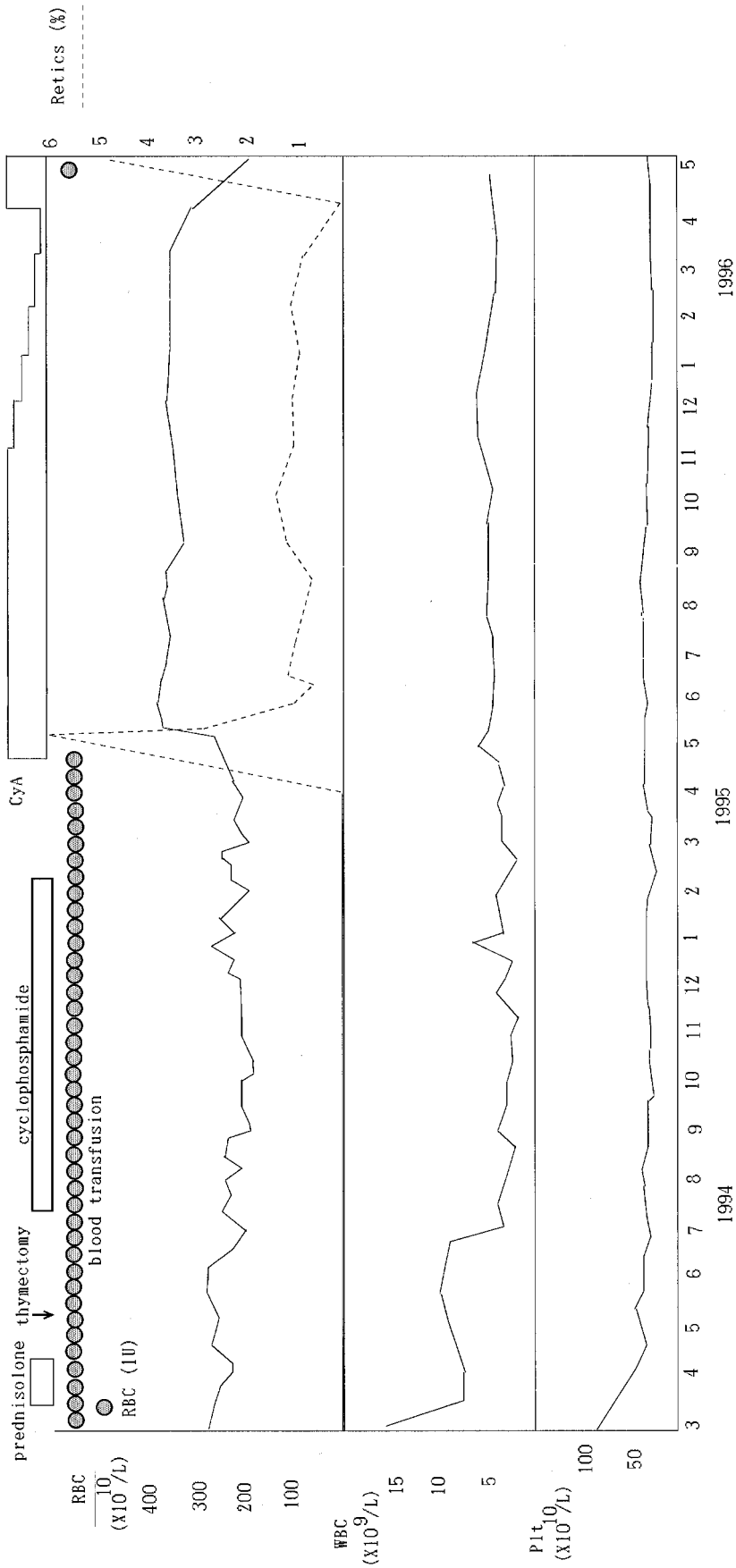

Fig. 3. Clinical course showing production of reticulocytes.

changed in this case. Nagashima et al. [12] reported a patient with thymoma and $CD8^+$ lymphocytosis. Theseinvestigators showed with Southern blotting that mononuclear cells of the peripheral blood and pleural effusion had the rearrangement of TCR-β. Yoshino et al. [13] analyzed T-lymphocytes from two patients with thymic tumors and showed that the numbers of γ- and δ- T cells were increased at the tumor site. Lishner et al. [14] showed that peripheral T cells from a patient with thymoma had the rearrangement of TCR-δ. Medeiros et al. [15] reported a patient with thymoma and T-cell lymphocytosis; their gene rearrangement study of peripheral blood revealed that TCR-β was in the germline configuration. Smith et al. [16] reported two cases of thymoma and T-cell lymphocytosis, in whom no monoclonal rearrangement of the the TCR-β genes in peripheral blood T cells was observed, although these cases were not accompanied by PRCA.

Most large granular lymphocytes (LGL) leukemia cases are clonal disorders with a rearrangement of TCR and are often associated with PRCA. Loughran [8] reviewed 129 cases of LGL leukemia and Oshimi et al. [9] 33 cases, but they did not describe an association between LGL leukemia and thymoma. In our case, since the morphology of lymphocytes was not LGL, our case of PRCA with thymoma might be a different clinical entity from LGL leukemia.

Masaoka et al. [17] analyzed 17 cases of PRCA with thymoma, and reported that the thymomas were characterized by spindle-type epithelial cells and lymphocyte invasion. However, they did not study the TCR rearrangement of thymoma. Handa et al. [18] reported a case of PRCA with thymoma and myasthenia gravis. These investigators demonstrated that T cells within the thymoma had a TCR-δ gene rearrangement, but they did not report that the peripheral T-cell lymphocytes were clonal. They postulated that the clonal thymic T cells secreted cytokines, which stimulated polyclonal peripheral lymphocytosis. There have been no reports concerning the monoclonality of both peripheral T cells and thymoma cells. We first reported here that both peripheral T cells and thymoma cells from a patient with PRCA had the rearrangement of TCR-β. As in LGL leukemia, PRCA with thymoma may be a clonal disorder of T lymphocytes. Since, as Masaoka et al. [19] reported surgical resection of thymoma had a limited effect (37.5%) on PRCA, the pathogenesis of thymoma in refractory cases might be considered invasion of T-cell clonal expansion. In reactive cases with thymectomy, the thymoma might be causative. In the 17 PRCA cases with thymoma studied by Masaoka et al. [17], two were complicated with myasthenia gravis and three with hypogammaglobulinemia. With these autoimmune and immunological complications, these cases might be considered a T-cell clonal disorder, as in our case. In previous studies, due to the low sensitivity of Southern blot analysis, the TCR gene rearrangement of thymus and peripheral blood T cells could not be demonstrated. We propose that some PRCA patients with thymoma have a T-cell clonal disorder, similar to some patients with LGL leukemia.

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